

Extraction and Cleanup of Sediments for Semivolatile Organics Following the Internal Standard Method

**Patricia Van Hoof and Jui-Lan Hsieh
Great Lakes Environmental Research Laboratory
National Oceanic and Atmospheric Administration
2205 Commonwealth Boulevard
Ann Arbor, MI 48105**

Standard Operating Procedure GLERL - M - 401 - 01

May 10, 1996

Version 1.0

Extraction and Cleanup of Sediments for Semivolatile Organics Following the Internal Standard Method

1.0 Scope and Application

This SOP is applicable to the extraction of semivolatile organic compounds from sediment matrices for analysis by gas chromatography (GC). Samples processed using this extraction method can be analyzed for semivolatile organic compounds listed in EPA Methods 608, 610, or 625, 8080, 8270, 8310 (EPA 1984, 1986). This method may be applied to other analytes once acceptable extraction efficiency has been demonstrated.

In this procedure, approximately 15-30 g wet weight sediment is extracted with dichloromethane (DCM) in a 30°C sonication bath. The extracts are dried over sodium sulfate and passed through a cleanup column. Column chromatography fractionation allows for separation of pesticides (PESs) from the majority of polychlorinated biphenyls (PCBs). Polyaromatic hydrocarbons (PAHs) elute along with the PESs. After a concentration step, internal standards are added and the sample is ready for analysis.

2.0 Responsible Staff

Project Manager: A Scientist responsible for 1) administration of the project; 2) providing project specific quality control requirements to the laboratory; 3) defending the data in a Quality Assurance Audit; and 4) reporting results to the client.

Laboratory Supervisor: A Technical Specialist or Scientist having expertise in the principles involved with this procedure and in the use of laboratory operations in general. Responsible for 1) ensuring that analysts are trained in the handling of solvents; 2) that appropriate quality control samples are included with the sample analysis to monitor precision and accuracy of target compound concentrations; 3) checking the analysts' work to ensure that samples are handled appropriately and that data are collected and interpreted correctly; 4) making decisions regarding problems with the analysis or deviations from the SOP; 5) defending the data in a Quality Assurance Audit; and 6) reporting results to project manager or client.

Analyst: A Technician, Technical Specialist, or Scientist assigned to conduct analyses using the procedure. Responsible for 1) understanding the proper handling of samples and solvents; 2) recording information regarding extractions and any deviations from the SOP in the appropriate log books; 3) analyzing the appropriate number of quality control samples for each batch of samples analyzed; 4) reporting results to the Project Manager; 5) participating in QA Audits.

3.0 Procedure

3.1 Apparatus and Reagents

3.1.1 Branson model 8210 ultrasonic cleaner with variable temperature water bath

- 3.1.2 Nitrogen evaporator, N-Evap or equivalent, heated with a water bath maintained at $35^{\circ}\pm 1^{\circ}\text{C}$
- 3.1.3 Rotary evaporator heated with a water bath maintained at $30^{\circ}\pm 1^{\circ}\text{C}$
- 3.1.4 Glassware
 - 3.1.4.1 250 mL Erlenmeyer flasks
 - 3.1.4.2 100 mL graduated cylinders
 - 3.1.4.3 1000 mL separatory funnels
 - 3.1.4.4 50 mL, 150 mL, and 250 mL beakers
 - 3.1.4.5 Glass rod, 20" long
 - 3.1.4.6 500 mL round bottom flasks
- 3.1.5 Apparatus for determining sample wet and dry weight
 - 3.1.5.1 Top-loading balance, accurate to 0.001 grams
 - 3.1.5.2 Drying oven maintained at 90°C
 - 3.1.5.3 Aluminum weighing pans
 - 3.1.5.4 Stainless steel spatulas
- 3.1.6 Long-tipped Pasteur pipettes (9.5" long)
- 3.1.7 Wide-bore Pasteur pipettes
- 3.1.8 Chromatography columns, 310 x 11 mm, with Teflon stopcocks
- 3.1.9 Drying oven maintained at 130°C
- 3.1.10 Desiccator
- 3.1.11 Muffle furnace
- 3.1.12 Sample vials, 2 mL capacity
- 3.1.13 Purified water (Millipore filtration system)
- 3.1.14 Saturated sodium chloride solution
- 3.1.15 Microliter syringes with replaceable needles

- 3.1.16 Glass wool, cleaned by soxhlet extraction using DCM
- 3.1.17 Sodium sulfate, anhydrous (Mallinkrodt- AR), heated to 450°C for at least 10 hrs, stored in 130°C oven
- 3.1.18 Silica gel (Aldrich grade 634, 100 - 200 mesh, 60 Å, 99+%), stored in a 130°C oven
- 3.1.19 Aluminum foil
- 3.1.20 Solvents: pesticide grade or equivalent
 - 3.1.20.1 Acetone (DMK)
 - 3.1.20.2 Methylene chloride (DCM)
 - 3.1.20.3 Hexane
 - 3.1.20.4 Diethyl Ether
- 3.1.21 Copper, activated by treating with concentrated HCl and washing with purified water twice, methanol twice, methylene chloride twice, and hexane twice, then storing in hexane.
- 3.1.22 Ring weights for securing flasks in water bath
- 3.2 Standards
 - 3.2.1 PAH surrogate standard - containing 2-4 mg/mL each of naphthalene-d₈, anthracene-d₁₀, benz(a)anthracene-d₁₂, benzo(e)pyrene-d₁₂, and benzo(ghi)perylene-d₁₂ in DMK.
 - 3.2.2 PAH internal standard - containing 20 mg/mL each of acenaphthylene-d₁₀, fluorene-d₁₀, chrysene-d₁₂, and perylene-d₁₂ in hexane.
 - 3.2.3 PCB surrogate standard - containing 400 ng/mL of PCB 14, 100 ng/mL PCB 65, and 80 ng/mL of PCB 166 in DMK.
 - 3.2.4 PCB internal standard - containing 170 ng/mL of PCB 30, and 130 ng/mL of PCB 204 in hexane.
 - 3.2.5 Matrix spiking standard - containing 2 mg/mL NIST SRM 1491 PAH standard mixture, 730 ng/mL total PCBs (Mullin's 1994 Aroclor mix), 660 ng/mL trans nonachlor, and 800 ng/mL of other individual pesticides in DMK.
- 3.3 Sample Handling

Samples shall be kept frozen at approximately -15°C until extraction, unless specified differently on a project-specific basis. All extracts held overnight shall be covered and refrigerated at 4°C. Extracts shall be saved and stored at 4°C in case re-analysis is required.

3.4 Labware Preparation

Prior to use, all glassware, Teflon, and other labware should be washed with hot, soapy water and rinsed with tap water, followed by purified water. Additionally, all non-volumetric glassware should be combusted in a muffle furnace @ 450°C for at least 16 hours. All volumetric glassware (i.e., graduated cylinders) should be solvent rinsed twice prior to use.

3.5 Dry Weight Determination

Dry-to-wet weight ratios of sediment samples should be analyzed in triplicate at the time of sediment sample extractions. Aluminum weighing pans are weighed, a sample of wet sediment is added, and the pans are weighed again. All samples are dried in the 90°C oven overnight or until a constant weight is obtained (no change in weight for a period of 3 minutes). Dry-to-wet weight ratios are determined as follows:

$$\frac{\text{Dry Wt}}{\text{Wet Wt}} = \frac{(g \text{ dry sample} + \text{pan}) - (g \text{ pan})}{(g \text{ wet sample} + \text{pan}) - (g \text{ pan})}$$

The dry weight of the sample to be extracted is determined as follows:

$$\text{Sample Dry Wt} = \text{Sample Wet Wt} \times \text{Mean Dry-to-Wet Wt Ratio}$$

Information for dry weight determinations shall be recorded in the Sediment Extraction Data Sheet (Attachment 1) for the individual samples. The following criteria for the coefficient of variation for four measurements were used: if dry/wet ratio $\leq 20\%$, then c.v. $< 25\%$
if dry/wet ratio $> 20\%$, then c.v. $< 15\%$.

3.6 Sample Extraction

Weigh the appropriate amount of well-mixed wet sediment onto a piece of tared alumina foil to the nearest 0.01 g. Fifteen to 30 g wet weight sediment shall be weighed out when trace (nanogram per gram dry weight) quantities of contaminants are expected. Mix sediment with appropriate amount of sodium sulfate and then scoop mixture into a 250 mL Erlenmeyer flask. One hundred microliters of the PAH surrogate standard and 62 microliters of the PCB surrogate standard are pipetted directly onto the sediment sample. Sample weights and standard volumes shall be recorded in the Sediment Extraction Data Sheet (Attachment 1).

Add 150 mL DCM to the flask, mixing thoroughly. Place ring weight around flask, and cover opening with foil. Place all batch samples in 30°C ultrasonic bath and sonicate for 60 minutes. After sonication, let stand in 30°C bath overnight (24 hours). The next day, sonicate samples again for 60 minutes at 30°C. Remove flasks from bath.

Set up filtration column as follows: plug the inside of a wide-bore Pasteur pipette with a piece of clean glass wool (3 cm in length). Clamp pipette filter above a 500 mL round bottom flask. Filter solvent phase from the Erlenmeyer flask through the glass wool via transfer Pasteur pipette.

3.7 Extract Evaporation

Reduce solvent in the flask to 15 mL using the rotary evaporator apparatus. Transfer the extract to a conical centrifuge tube and further reduce solvent to approximately 1 mL using the nitrogen evaporator apparatus. Exchange solvent by adding 5 mL hexane, then evaporate to approximately 1 mL and repeat this step twice more. Transfer extract from the centrifuge tube to a pre-combusted 2 mL sample vial, rinse with 1 mL hexane, add to vial, and cap securely.

3.8 Pre-Column Preparation and Extract Clean-up

3.8.1 Preparation of 3% deactivated silica gel and 10% deactivated alumina

Day 1

Place silica in a 130°C oven overnight (at least 18 hrs). Place alumina in a shallow ceramic dish and activate it in a muffle furnace @ 450°C for at least 16 hours.

Day 2

Remove silica and alumina from oven and let cool on counter top until room temperature is reached (approximately 5 - 10 minutes). When silica and alumina have reached ambient temperature, deactivate it as follows:

--Working quickly, weigh out desired amount of silica and alumina in separate round-bottom flasks. Stopper immediately.

--Add 3% and 10% weight/volume of deionized water to silica and alumina respectively, using the following equation:

$$\frac{\% \text{ deactivation}}{100 - \% \text{ deactivat.}} = \frac{\text{mL DI water}}{\text{weight of silica (gm)}}$$

--Shake for 10 minutes. Store in desiccator overnight for equilibration. Use deactivated silica and alumina within three days. Any unused silica and alumina may be reused after re-activating and re-deactivating.

3.8.2 Preparation of Sodium Sulfate

Pour aliquot of sodium sulfate into a shallow ceramic dish. Heat at 450°C for 16 hrs. Cool, then store in 130°C drying oven until ready for use.

3.8.3 Column Preparation

Assemble stopcock on column. Stuff glass wool plug (approximately 1 cm) into lower end of the column with a glass rod. Clamp column securely onto stand. Place empty 150 mL beaker under column. Close stopcock; fill column half full with hexane. Make a slurry of hexane and 3-grams deactivated silica gel. Open stopcock to partially drain excess hexane dislodging any trapped bubbles in glass wool plug.

Pour slurry into column tapping column gently with a glass rod or spatula. Rinse column and beaker with hexane via Pasteur pipette. Make a slurry of hexane and 10 grams of deactivated alumina. Pour slurry into column. Cap column with 2 inches sodium sulfate. Wash column with 25 mL hexane for equilibration. When hexane level reaches 1 cm above top of sodium sulfate, close stopcock to prevent further dripping. Never let column run dry. If column is not going to be used immediately, cover column and tip with foil.

3.8.4 Extract Clean-Up

First fraction: PCBs, HCB, 4,4'-DDE, aldrin, and heptachlor

Sonicate sample for 10 seconds before loading onto column. Open stopcock and let drip until hexane level is at the top of the sodium sulfate. Place a conical centrifuge tube under the column to catch eluate of first fraction. Measure 35 mL of hexane into a graduated cylinder. Load sample onto column via Pasteur pipette. Open stopcock and let sample flow to just the top of the sodium sulfate. Rinse sample vial with approximately 5-mL hexane in the cylinder and load onto column. Set drip rate to approximately 2 drops per second. When rinse reaches the top of the sodium sulfate, add the rest of hexane to the column.

Second fraction: Alpha-BHC, gamma-BHC, heptachlor epoxide, alpha-chlordane, gamma-chlordane, trans-nonachlor, dieldrin, cis-nonachlor, 4,4'-DDT, 4,4'-DDD and all PAHs.

While the hexane is dripping from the first fraction, measure 50 mL of 10% diethyl ether in hexane into the graduated cylinder. When the hexane level from the first fraction reaches the top of the sodium sulfate layer, add the 10% solution to the column. Replace conical centrifuge tube with another for collection of the second fraction. Once the column has stopped dripping, remove the tube and evaporate the solvent.

3.8.5 Evaporation of First and Second Solvent Fractions and Sample Preparation

Place the centrifuge tubes from the first and second fractions in the nitrogen evaporator water bath. Reduce solvent in the flasks to 1 mL using the nitrogen evaporator apparatus. The second fraction requires solvent exchange into hexane by adding 5 mL hexane. Rinse sample tubes with a small amount of hexane and transfer to sample vials, keeping volume of solvent less than 1 mL. Add the appropriate amount of internal standards. Record the amount and ID of the IS in the Sediment and Tissue Extraction Data Sheet. The first fraction requires sulfur cleanup by adding activated copper beads. Add more if all the copper beads in sample vial turn black. Cap sample vials, mix, and store at -15°C (first fraction samples) or at 4°C (second fraction samples) until gas chromatography analysis.

4.0 Quality Control Sample Frequency

Samples prepared using this procedure should be processed in batches of approximately 10 samples plus quality control samples.

Laboratory Reagent Blank (LRB) - analyze one per batch. Prepare by working through the sample procedure without a sample matrix, but including Na₂SO₄, extraction solvents, and appropriate surrogates and internal standards.

Laboratory Matrix Spike (LMS) - analyze one per batch. Prepare by fortifying a matrix known to be below detection limits for an analyte(s) (e.g. pre-industrial sediment) with known amount of surrogates, target analyte(s), and internal standards.

Laboratory Environmental Matrix Blank (MSB) - analyze less frequently, once or twice per field trip. Prepare by working a matrix known to be below detection limits for an analyte(s) through the sample procedure including surrogate and internal standard additions.

Laboratory Duplicate (LD1) - analyze one per batch. Prepare by splitting a sample and treating identically throughout the analytical procedure.

Laboratory Performance Check (LPC) - run one per batch. A calibration solution used to verify whether the initial calibration data are currently valid.

Field Duplicate (FD1) - analyze one per batch if possible, otherwise as often as logistics allow.

Field Reagent Blank (FRB) - collect one per field trip. Collect sediment of pre-industrial age for subcores from several sites (~ 7). This “clean” older sediment will then be run through the entire collection and analytical systems just as routine field samples.